AMENDMENTS

In the Claims:

1. (Currently Amended) A process for producing a stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, said process comprising:

a) redundantly labeling said control cell ~~with at least two distinct fluorescent labels having the same spectral properties~~ with at least two fluorescent labels having different emission spectra wherein said spectra are detected through the same spectral window to ensure quantification of an intact control cells;

b) permeabilizing said control cell;

c) contacting said labeled cells with a cell fixative said fixative effecting stabilization of both cellular structure and antigenic moieties present on said control cells;

d) subsequently removing the excess fixative to promote long-term storage of said control cells, said control cells being physically and biologically stable for at least six months wherein said physical and biological stability provides efficient immunomagnetic recovery in rare cell analysis.

2. (Original) The process as claimed in claim 1, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

3. (Original) The process as claimed in claim 1, wherein said fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

4. (Original) The process as claimed in claim 1, wherein said control cells are labeled with an antibody immunologically specific for an antigen present on said cells, said antibody being conjugated to a fluorescent molecule.

5. (Cancel)

6. (Cancel)

7. (Currently Amended) A process for producing a stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, said process comprising:

a) redundantly membrane labeling said control cell ~~with at least two distinct fluorescent labels having the same spectral properties~~ with at least two fluorescent labels having different emission spectra wherein said spectra are detected through the same spectral window to ensure quantification of an intact control cells;

b) permeabilizing said control cell;

c) contacting said labeled cells with a cell fixative said fixative effecting stabilization of both cellular structure and antigenic moieties present on said control cells;

d) subsequently removing the excess fixative to promote said long-term storage of control cells, said control cells being physically and biologically stable for at least six months, wherein said control cell expresses epithelial cell adhesion molecule (EpCam) on its surface and also expresses cytokeratin intracellularly.

8. (Original) The process as claimed in claim 7, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

9. (Currently Amended) The process as claimed in claim 7, wherein said label ~~membrane labeling~~ is selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

10. (Currently Amended) A stabilized cell, permeabilized, for use as an internal control in methods for isolating and identifying rare cells, said control cell having determinants in common with said rare cells, wherein said control cell is labeled redundantly ~~with at least two distinct fluorescent labels having the same spectral properties~~ with at least two fluorescent labels having different emission spectra wherein said spectra are detected through the same spectral window to ensure quantification of an intact control cells, and cellular components and antigenic moieties of said control cell have been stabilized for at

least six months by exposure to fixative wherein said stability provides efficient immunomagnetic recovery in rare cell analysis.

11. (Currently Amended) The control cell as claimed in claim 10, suspended in a buoyant density medium, having the same density as said control cells wherein said control cell does not gravity settle.

12. (Original) The control cell as claimed in claim 10, wherein said ~~cell~~ fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

13. (Original) The control cell as claimed in claim 10, wherein said fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

14. (Original) The control cell as claimed in claim 10, wherein said control cells are labeled with an antibody immunologically specific for an antigen present on said cells, said antibody being conjugated to a fluorescent molecule.

15. (Cancel)

16. (Currently Amended) A stabilized cell, permeabilized, for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, and comprising a detectably labeled membrane, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being effected by exposure to a fixative, wherein said control cell is a cultured tumor cell expressing EpCam on its surface and cytokeratin intracellularly wherein said stabilization does not alter lightscattering and autofluorescent properties of said detectable label on said control cell.

17. (Currently Amended) The control cell as claimed in claim 16, suspended in a buoyant density medium, having the same density as said control cells wherein said control cell does not gravity settle.

18. (Original) The control cells as claimed in claim 16, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

19. (Original) The control cell as claimed in claim 16, wherein said membrane label is selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

20. (Currently Amended) The control cell of 16, wherein said membrane is redundantly labeled ~~with at least two distinct fluorescent labels having the same spectral properties~~ with at least two fluorescent labels having different emission spectra wherein said spectra are detected through the same spectral window to ensure quantification of an intact control cells.

21. (Currently Amended) The control cell as claimed in claim 16, said cell being ~~an~~ a cultured SKBR3 breast cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu.

22. (Currently Amended) The control cell as claimed in claim 16, said cell being ~~an~~ a cultured MCF-7 breast cancer cell, further comprising a second detectably labeled surface determinant which is an estrogen determinant.

23. (Original) The control cell as claimed in claim 16, said cell being an LNCaP prostate cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA, and androgen receptor.

24. (Cancel)

25. (Cancel)

26. (Cancel)

27. (Original) The control cell as claimed in claim 16, said cell being a C32 melanoma cancer cell, further comprising a second detectably labeled surface determinant which is a CD146 molecule.

28. (Currently Amended) A stabilized cell, permeabilized, for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, and comprising a redundantly labeled membrane, said membrane being labeled with at least two distinct fluorescent labels having the same spectral properties to ensure quantification of an intact control cells, said

cells further comprising stabilized cellular components and antigenic moieties, said stabilization being effected by exposure to a fixative, wherein said control cell is selected from the group consisting of tumor cells, bacterially infected cells, virally infected cells, myocardial cells, and endothelial cells in circulation, and fetal cells in maternal circulation, wherein said stability provides efficient immunomagnetic recovery in rare cell analysis.

29. (Currently Amended) The control cells as claimed in claim 28 ~~27~~, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

30. (Currently Amended) The control cell as claimed in claim 28 ~~27~~, wherein said fluorescent label is a membrane label selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

31. (Currently Amended) The control cell as claimed in claim 28 ~~27~~, wherein said control cells are labeled with an antibody immunologically specific for an antigen present on said cells, said antibody being conjugated to a fluorescent molecule.

32. (Cancel)

33. (Cancel)

34. (Currently Amended) The control cell as claimed in claim 28 ~~27~~, suspended in a buoyant density medium.

35. (Currently Amended) An improved method for detecting and enumerating rare cells in a mixed cell population, the presence of said rare cells in said population being indicative of severity of a disease state, comprising:

a) obtaining a blood sample from a test subject, said sample comprising a mixed cell population suspected of containing said rare cells;

b) preparing an immunomagnetic sample wherein said blood sample is mixed with colloidal magnetic particles between 90 nm and 200 nm in size coupled to a ligand which reacts specifically with a determinant of the rare cells, to the substantial exclusion of other sample components after exposure to an externally applied magnetic field;

c) contacting said immunomagnetic sample with at least one reagent which labels a determinant of said rare cells; and

d) analyzing said labeled rare cells to determine the presence and number of any rare cells in said immunomagnetic sample, the greater the number of rare cells present in said sample, the greater the severity of said disease state, wherein the improvement comprises the addition of a stabilized cell, permeabilized, for use as an internal control cell in said method, said control cell having determinants in common with said rare cells and wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cell have been stabilized for at least six months by exposure to fixative.

36. (Currently Amended) The method as claimed in claim 35~~34~~, wherein said rare cell is a cancer cell and said disease state is cancer.

37. (Currently Amended) The method as claimed in claim 35, wherein said membrane is redundantly labeled ~~with at least two distinct fluorescent labels having the same spectral properties~~ with at least two fluorescent labels having different emission spectra wherein said spectra are detected through the same spectral window to ensure quantification of an intact control cells.

38. (Original) The method as claimed in claim 35, wherein said membrane label is selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

39. (Currently Amended) The method as claimed in claim 35~~34~~, wherein said ligand is an anti-EpCam, and said reagent labels an intracellular cytokeratin, said EpCam and said cytokeratin being present in both said rare cell and said control cell.

40. (Currently Amended) The method as claimed in claim 39~~38~~, wherein the control cell is an SKBR3 breast cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu.

41. (Currently Amended) The method as claimed in claim 39~~38~~, wherein the control cell is MCF-7 breast cancer cell, further comprising a second detectably labeled surface determinant which is an estrogen determinant.

42. (Currently Amended) The method as claimed in claim 39~~38~~, wherein the control cell is an LNCaP prostate cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA, and androgen determinant.

43. (Cancel)

44. (Cancel)

45. (Cancel)

46. (Currently Amended) The method as claimed in claim 39~~38~~, wherein the control cell is a C32 melanoma cancer cell, further comprising a second detectably labeled surface determinant which is a CD146 molecule.

47. (Cancel)

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52. (Cancel)

53. (Cancel)

54. (Cancel)

55. (New) An improved kit for screening a patient sample for the presence of circulating tumor cells having coated magnetic nanoparticles with a protein based coating material and anti-EpCAM coupled, directly or indirectly, to said base coating material, at least one antibody having binding specificity for a cancer cell determinant, cell specific dye for excluding sample components other than said tumor cells from analysis wherein said improvement comprises:

a) a container having stabilized control cells, permeabilized, for use as an internal control, said stabilized control cells having determinants in common with said

tumor cells, wherein said membrane of said control cell is detectably labeled, and cellular components and antigenic moieties of said control cells have been stabilized for at least six months by exposure to fixative to maintain lightscattering and autofluorerscent properties; and

b) a buoyant density medium wherein said stabilized cells are in suspension within said container.

56. (New) The kit as claimed in claim 55, wherein the control cell is a cultured SKBR3 breast cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu.

57. (New) The kit as claimed in claim 55, wherein the control cell is a MCF-7 breast cancer cell, further comprising a second detectably labeled surface determinant which is an estrogen receptor.

58. (New) The kit as claimed in claim 55, wherein the control cell is an LNCaP prostate cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA, and androgen determinant.

59. (New) The kit as claimed in claim 55, wherein the control cell is a C32 melanoma cancer cell, further comprising a second detectably labeled surface determinant which is a CD146 molecule.

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